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PHYSIOLOGICAL STUDIES OF EFFECTS OF FORMALDEHYDE ON WHEAT[1]

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(WITH TWELVE FIGURES)

Introduction

Copper sulphate and formaldehyde have been most commonly used as fungicides in the treatment of seed wheat. The choice between the two has often been determined by local custom and prejudice, while in other cases climatic differences have been thought to be worthy of consideration in the selection. During the twenty years, approximately, that formaldehyde has been used as a dip or spray, as a gas (**44, 53**) or with steam (**39**), reports have differed radically in the degree of favor with which it has been viewed.

One group of experimenters has reported injury to germination or seedling vitality, or both, following the use of formaldehyde. STEPHENS of the Sherman County Branch Experiment Station at Moro, Oregon, has consistently reported injury in his station reports since 1913 (**50**). In 1917 he noted that 18.5 per cent of the seed wheat was killed. He has made the further important observation that in many cases seedlings may progress in development but with lessened vegetative vigor. HEALD and WOOLMAN (**31**) found germination reductions at concentrations of 30–40 gallons of water

[1] The project on which the present paper is based was financed under the Adams Fund, to which acknowledgment is hereby made.

to the pint of formaldehyde. In Utah, STEWART and STEPHENS (52) noted vitality reductions in wheat, barley, and oats, but thought the advantages outweighed the injury. MACKIE (37) of California noted that seed stored after treatment uniformly showed poor germination. Even with proper drying the tissues appeared hardened, causing retardation and distortion of the young seedlings. Varying degrees of injury have been reported by many different investigators (14, 19, 20, 21, 22, 53, 57, 58).

On the other hand, formaldehyde has been approved in varying measures by different investigators, some of whom recognized the dangers and injury in some cases, but have felt that the advantages outweighed the injury. The War Emergency Board of American plant pathologists found little injury from formaldehyde except when the concentrations were higher than the usual 1 part to 320 parts of water, or when the other common precautions in treatment had not been observed. This work was based on the reports of seventeen experiment stations, and is probably the most complete and uniformly secured set of data available from so large an area of country (35). Many other workers in America and Europe have reported in similar vein (6, 16, 32, 36, 38, 40, 45, 51, 55).

Within the past two years two most interesting papers have appeared, in which the possibilities of avoiding injury from formaldehyde treatment have been suggested. BRAUN (13) finds the injury apparently much diminished by not treating the grain until imbibed with water. It is believed that exterior disinfection is thus attained, and a much less amount of formaldehyde enters the grain under these conditions. Miss HURD (33) believes that when seeds are treated in formaldehyde and subsequently allowed to dry, the polymer paraformaldehyde is deposited on the seed coat with serious eventual injury. Here, instead of the "pre-soak," we have the recommendation of washing in water subsequent to treatment to avoid the harmful paraformaldehyde deposits. With the work of Miss HURD there appears to be a better explanation than formerly of the source of the injury of formaldehyde to seeds. Amid a wide diversity of opinions as to the value of the disinfectant, and with differing recommendations for reduction in treatment injury, it seemed altogether desirable that something be learned as

to the exact nature of the effect exerted by formaldehyde on the physiological processes of seeds, as shown by wheat. Accordingly, the Oregon Experiment Station has been occupied in such studies, during parts of the past three seasons, and inasmuch as local conditions have necessitated the temporary discontinuance of this work, it was thought well to report the results already obtained.

Experimentation

Wheat for these studies was kindly furnished by Mr. STEPHENS of the Oregon Branch Experiment Station. In order that the behavior of this wheat in relation to formaldehyde might be known, it was thought advisable first to determine the effect on germination of varying the concentration and also the time of treatment. The formaldehyde used was the ordinary commercial material, the strength of which was determined according to the method outlined by HAYWOOD and SMITH (30), and found to contain 39.3 parts per hundred by volume of the formaldehyde gas.

In the studies of the effect of varying the time of treatment, the period was varied from 5 to 300 minutes of soaking in formaldehyde 1–320. The number of seeds used was 10,800, one-third being grown in blotters in the customary manner, one-third in soil in porous clay germinators indoors, and one-third outdoors in pots of soil exposed to the weather and a temperature between 40° and 60° F. The indoor samples were grown in the laboratory, and, as might be expected, germination was much more prompt at the higher temperature. It was found that the time of dip between twenty and forty minutes only slightly reduced the germination percentages. A somewhat greater drop in the curves (figs. 1, 2) occurs as the time is lengthened up to four hours, although the drop is not great in most cases. The seeds germinated in soil displayed a somewhat greater percentage of injury, as measured by appearance above soil, than was true of the samples grown in blotters. This difference between the behavior of formaldehyde treated seeds when germinated in soil and in blotters was noted by CRANEFIELD (20) in studying the effect of the fungicide on oats. He found the injury in oats grown in the soil averaged four times greater than that of seed grown in blotters. The explanation of this difference in

apparent injury was given by WALLDEN (56), who thought injuries to the coleoptile, making it difficult to pierce the soil, do not prevent the germination of seeds in blotters. Miss HURD (33), after making a similar observation, expresses preference for the blotter studies, which she believes show more clearly the distortion incident to

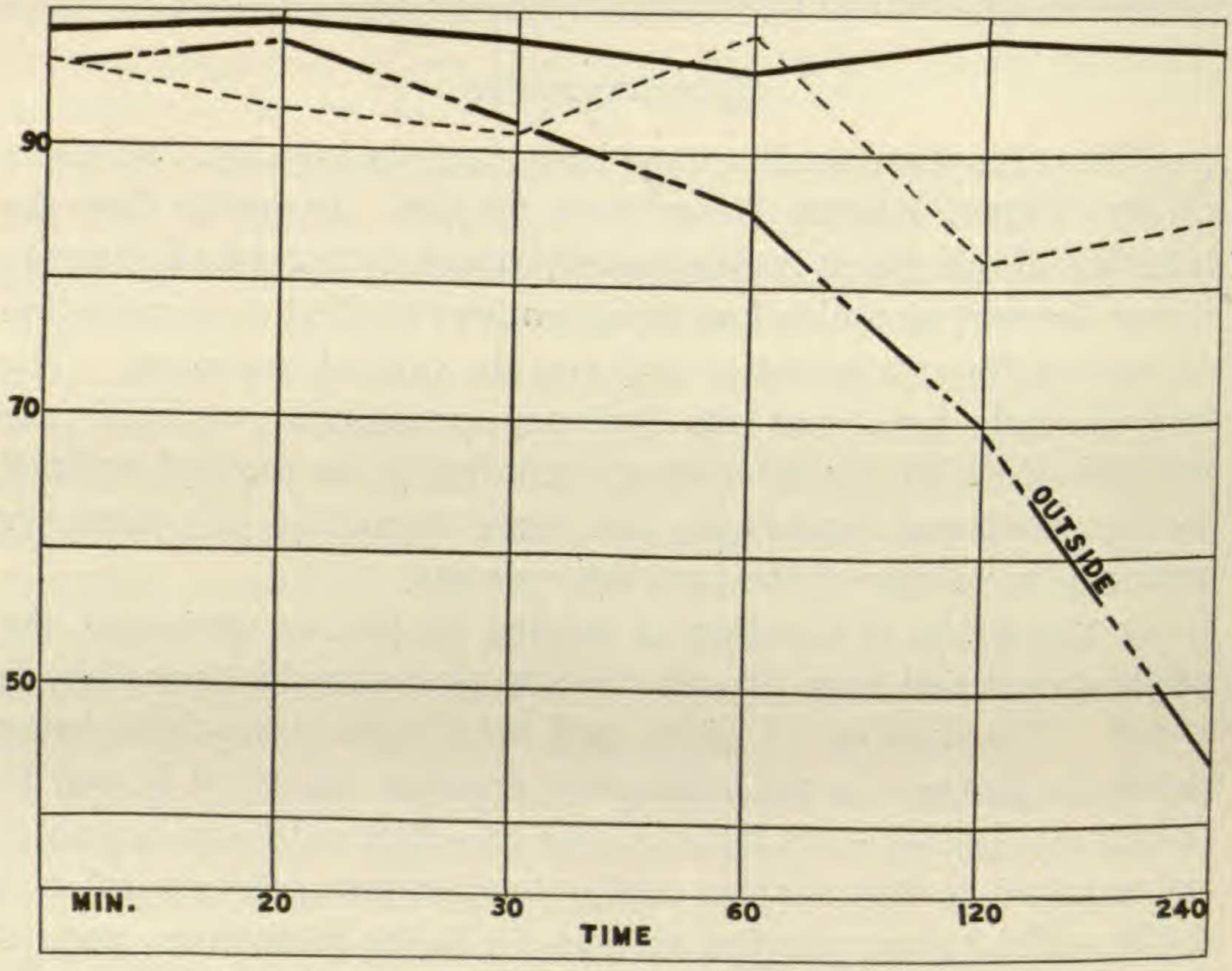

FIG. 1.—Effect of time of soaking wheat on percentage germination, Hybrid 128 wheat from Moro, Oregon: solid line shows percentage germination in blotters, broken lines in soil indoors and outside; formaldehyde mixed 1–320 parts of water; summary of 3600 seeds tested.

injury, even though the percentage stand which would be attained under field conditions by this method could only be estimated.

In varying the concentrations of formaldehyde, treatment was for ten minutes at 20° C., and the concentrations were varied from 40 to 320 parts of water to 1 part of formaldehyde. Uniform dropping in germination occurred in all cases with increasing concentrations. As compared with the water dipped controls, there was little injury apparent at the usual concentration of 1–320; but with a concentration of 1–160 the germination curves began to

fall, and at 1–40 the germination was cut from 40–60 per cent, both in the blotters and in the soil. Here again, as in the previous series, the injury was greatest in the outdoor soil, less in soil indoors, and least of all in the blotter tests (figs. 3, 4).

Formaldehyde readily forms various polymers (**8, 25**). On standing in the cool a flocculent white precipitate forms readily,

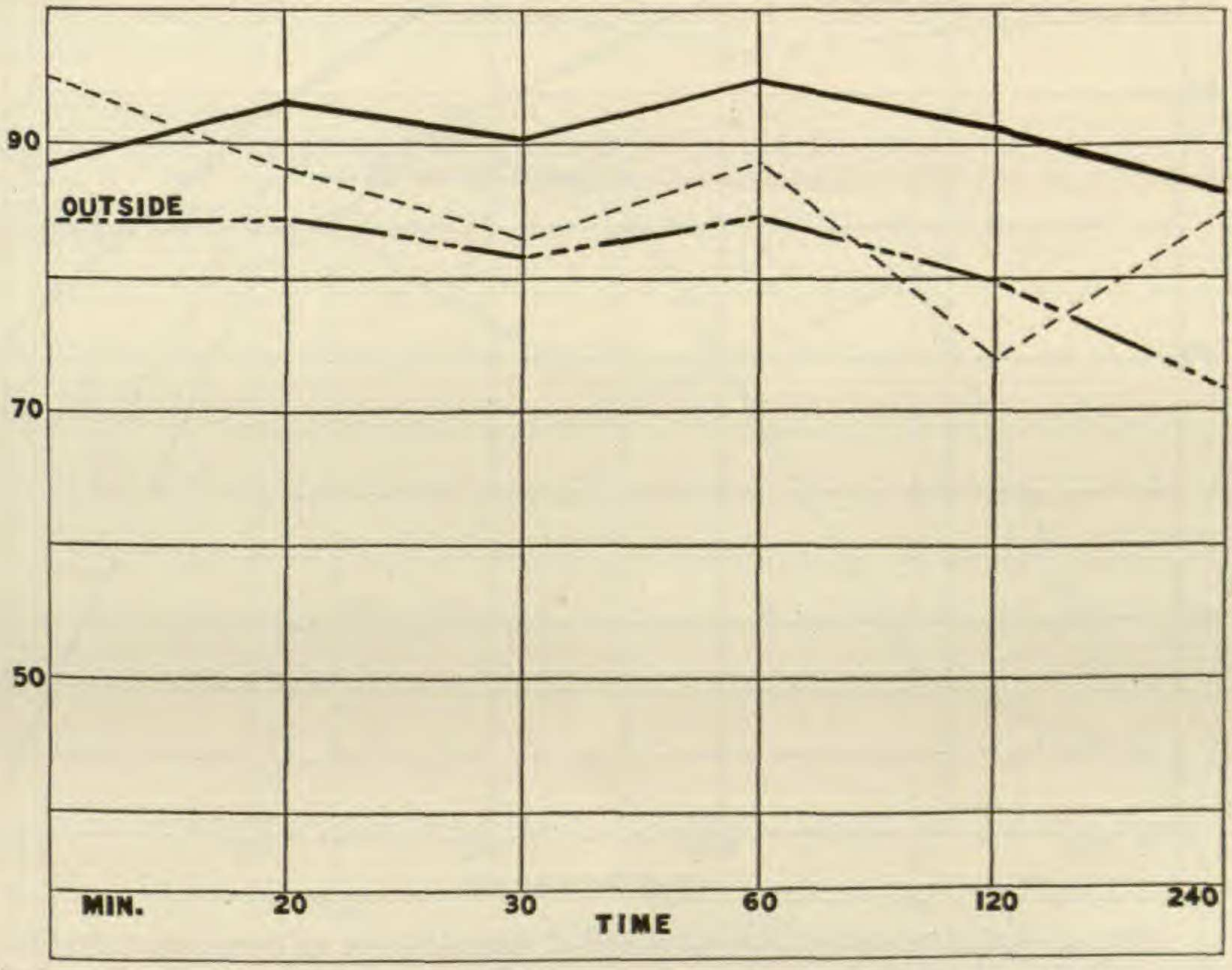

FIG. 2.—Effect of time of soaking wheat on percentage germination, Turkey Red wheat from Moro, Oregon: solid line shows percentage germination in blotters, broken lines in soil indoors and outside; formaldehyde mixed 1–320 parts of water; summary of 3600 seeds tested.

or on concentration of the commercial solutions. This is ordinarily referred to as paraformaldehyde, although the various polymers are probably often found more or less associated, and means for the identification of the various forms are not well known. Efforts have been expended toward developing methods to prevent such polymerization (**28**), but these methods have not been adopted in general. If wheat is dusted with the white flakes of this so-called paraformaldehyde, serious injury results. Turkey Red so treated

gave in one series of tests 9.5 per cent germination in blotters and 15 per cent in soil, as compared with 93.5 and 93 per cent respectively for the controls. This effect of the white polymer on the grain was noted by COONS and MCKINNEY (**19**), who found that it does not readily air out of grain but persists on it, so that its pres-

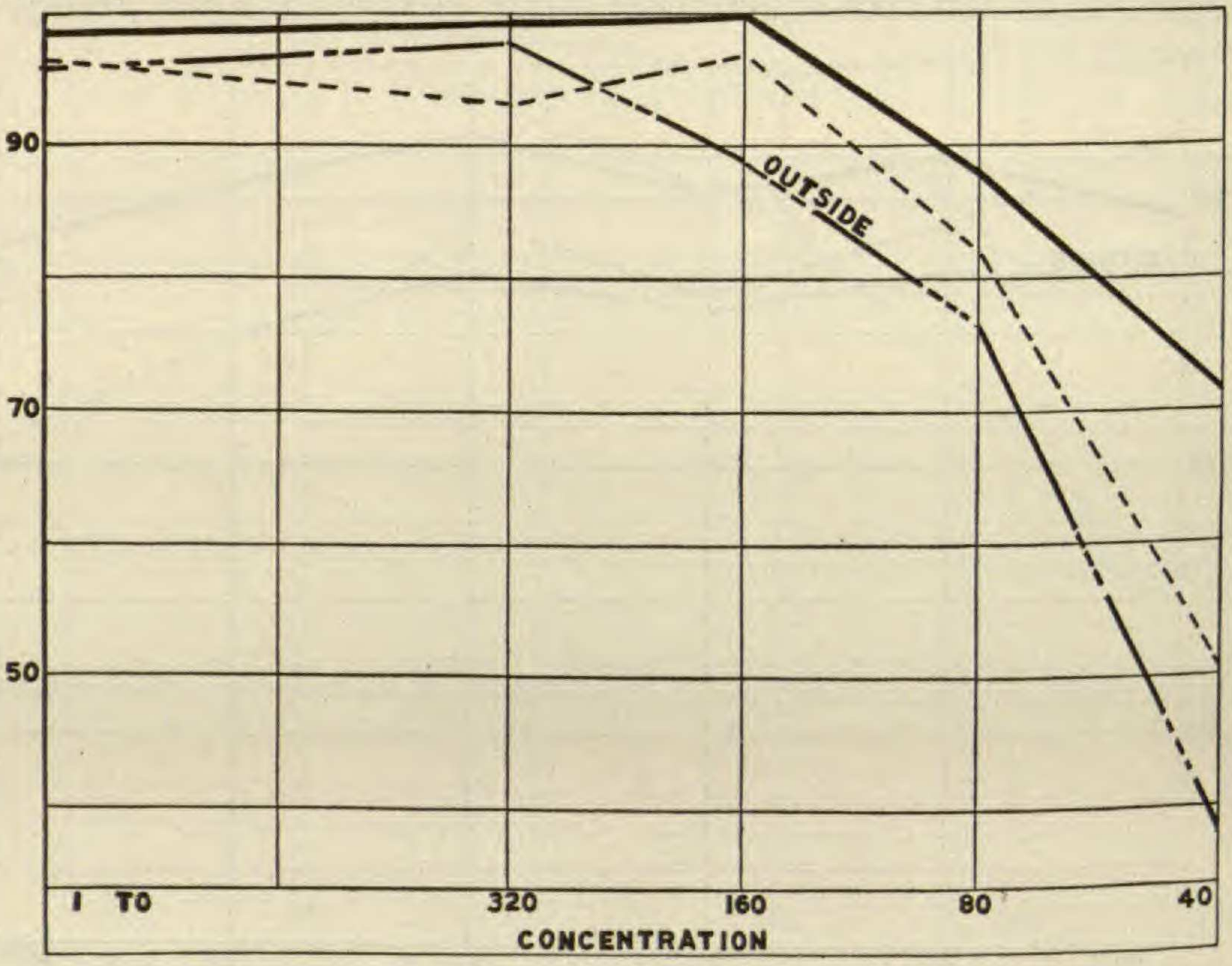

FIG. 3.—Effect of varied concentrations of formaldehyde on percentage germination, Hybrid 128 wheat from Moro, Oregon: solid line shows percentage for seeds germinated in blotters, broken lines for seeds in soil outside and indoors; ten minutes of soaking; summary of 3000 seeds tested.

ence could be demonstrated by an indicator after the grain had been exposed to the air of the laboratory for many months. Miss HURD (**33**) later has emphasized the extreme importance of the polymer as the possible channel through which injury from formaldehyde ordinarily results.

PERMEABILITY

It was recognized that it must be determined whether formaldehyde actually penetrates the coat of wheat. It has long been known that the seed coats of many seeds exhibit varying powers of exclu-

sion. BROWN (15) showed this to be conspicuously the case for barley, while SHULL (49) found semipermeability of seed coats a rather general situation. SCHROEDER (47) showed that the coat of wheat is permeable to the entry of mercuric chloride, iodine, alcohol, ether, chloroform, and acetic acid when in water solutions. Injury to the seed coat destroys this seed coat power of exclusion.

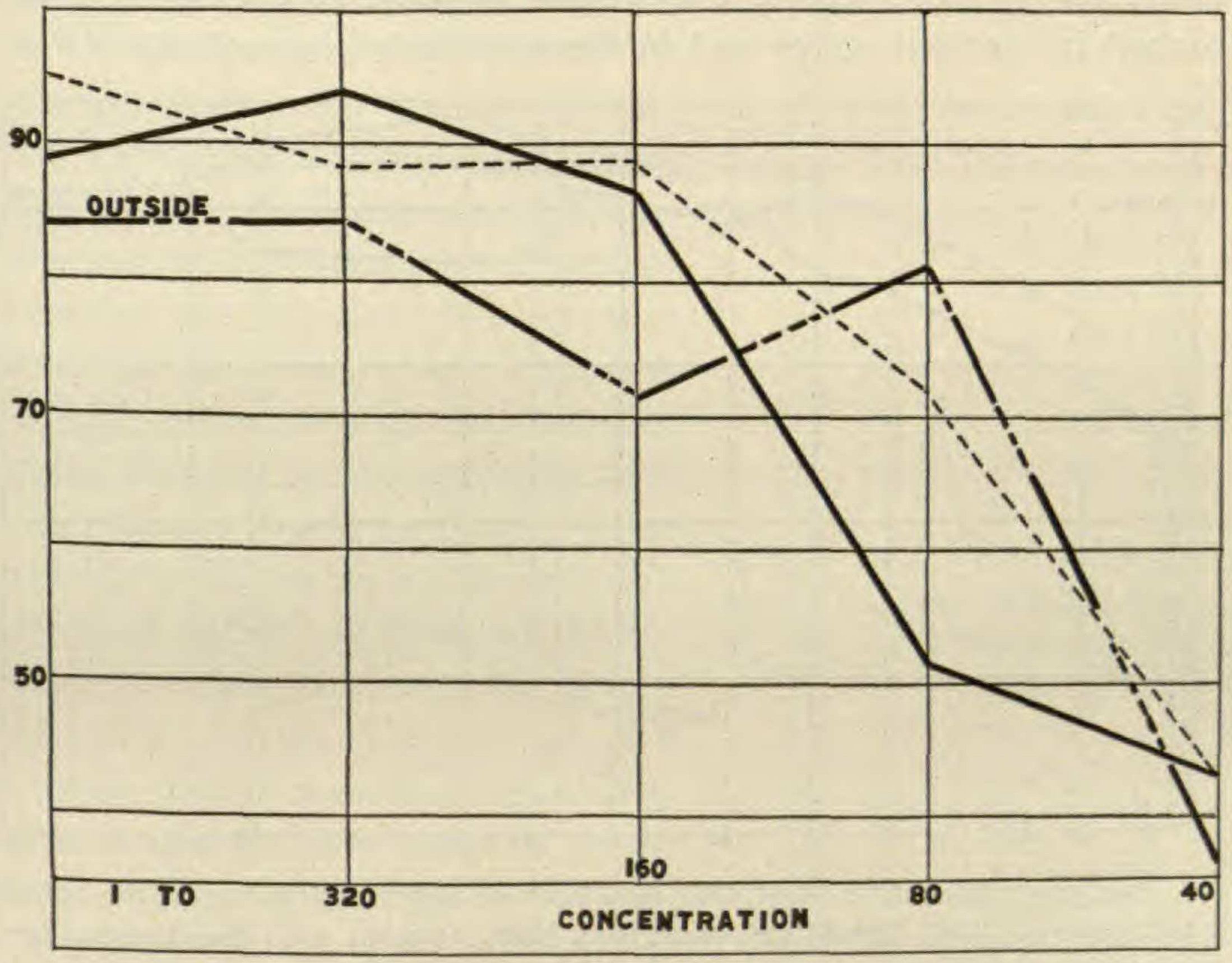

FIG. 4.—Effect of varied concentrations of formaldehyde on percentage germination, Turkey Red wheat from Moro, Oregon: solid line shows percentage for seeds germinated in blotters, broken lines for seeds in soil indoors and outside; ten minutes of soaking; summary of 3000 seeds tested.

Miss HURD (34) found that injuries from the entry of fungicides are much worse when seed coat cracks exist over the embryo.

Two methods were employed in studying the relation of the seed coat of wheat to formaldehyde entry. After various difficulties in technique, at the suggestion of Dr. E. M. HARVEY, the method was finally adopted of sealing the seeds one at a time to the end of small glass tubing, into which formaldehyde solution was placed. After allowing the seed to be in contact with the solution

for 3–4 days, the dry tip of the grain exterior to the tube was sectioned and treated directly with the Schryver formaldehyde reagent (29). With long periods of exposure to high concentrations of formaldehyde (1–8) penetration appears to be possible at either tip of the grain or on either face. The second method employed was to measure the degree of semipermeability of the seed coat indirectly by determining the weight increase of the seeds when soaked in distilled water and in formaldehyde respectively. For-

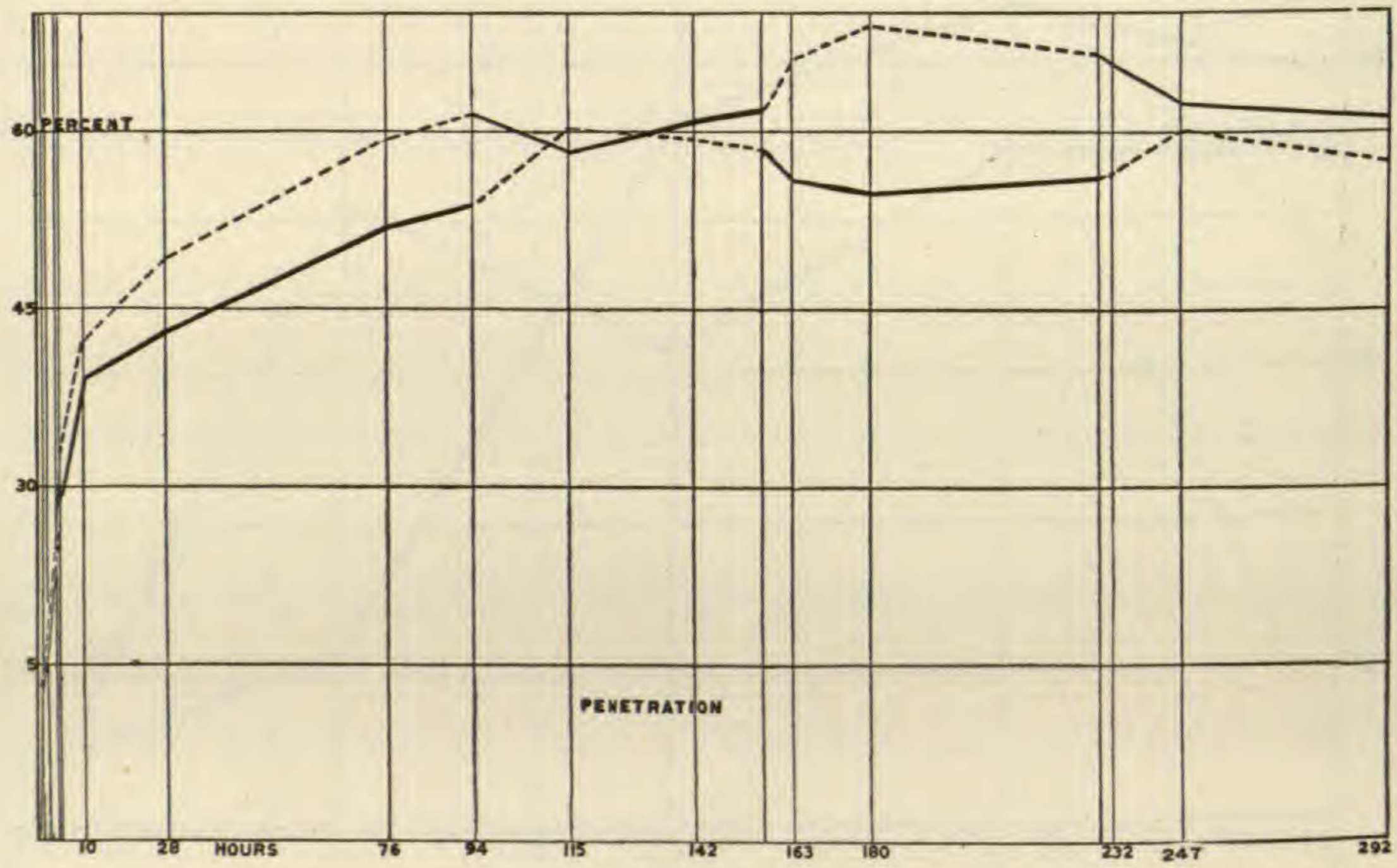

FIG. 5.—Comparative absorption by wheat of water and formaldehyde mixed 1 to 8 parts of water, Hybrid 128 wheat from Moro, Oregon: solid lines indicate percentages of weight increases when seeds were soaked in formaldehyde, broken lines when soaked in water.

maldehyde of high concentration (1–8) was used, in order to make more conspicuous any semipermeability differences of the coat toward the water and formaldehyde. In harmony with the results of BROWN (15), if seeds with semipermeable coats be placed in salt solutions, water will be taken up by imbibition until the inward force is offset by the equal outward osmotic force incident to the solution outside. If the two forces just balance each other, and if the coats be perfectly semipermeable, further soaking of the seeds in solution will not cause a rise in the curve indicating percentage weight increase, and the curve will continue horizontal. On the

other hand, if there is a gradual entry of the salt into the seed, the curve will flatten out quickly, and after changing the seeds back into pure water, the sudden and extensive rise of the curve as the water enters will be followed by a subsequent sinking as the salt gradually diffuses out of the seed again. In this way, by long and careful soaking of seeds in water and solutions of higher osmotic concentration, it is possible to determine whether the substance in solution is entering the seed coat. Frequent weighings are essential, each seed lot being dried of surface moisture with filter paper at each weighing. After the curves approach equilibrium, the seeds in either solution are transferred to the other one, with special attention to the behavior of the curves at this reversal of solutions. The weighings were made in triplicate with 3.5 gm. samples. Seventeen intervals, covering 292 hours of soaking, were followed by computation of percentages. A study of the resultant curves (fig. 5) leads to the conclusion that formaldehyde slowly penetrates the seed coat, and that when the grain is again transferred to distilled water, the formaldehyde gradually diffuses outward. These results are shown in table I. The variations at the time of reversal are by no means so great, even with this high concentration of formaldehyde,

TABLE I

DATA ON PENETRATION AS SUMMARIZED IN FIG. 5; PERCENTAGE WEIGHT INCREMENTS AT VARYING PERIODS OF SOAKING OF WHEAT

No.	Air dry weight	Hours of soaking																
		1	2	4	4.75	10	28	76	94	115	142	157	163	180	229	232	247	292
1	3.5048	**11.9**	**15.7**	**22.9**	28.9	**38.5**	**43.4**	**53.2**	**55.1**	61.4	60.0	60.1	**57.3**	**55.4**	**57.1**	57.8	61.0	58.0
2	3.478	**11.9**	**15.6**	**23.6**	29.2	**39.2**	**43.4**	**51.6**	**53.3**	60.8	60.0	58.9	**56.4**	**54.7**	**56.5**	56.5	60.6	58.2
3	3.4106	**12.3**	**15.9**	**24.2**	30.1	**40.7**	**44.1**	**52.3**	**54.5**	60.0	59.5	58.6	**55.5**	**55.1**	**55.7**	57.1	59.8	57.2
4	3.2846	13.0	17.5	28.0	**32.8**	42.5	50.1	60.8	62.6	**59.5**	**62.1**	**63.1**	68.3	70.5	68.6	**66.4**	**63.2**	**61.4**
5	3.5406	13.3	18.1	27.9	**32.7**	42.9	49.6	59.4	61.6	**58.8**	**60.4**	**61.5**	65.9	68.9	64.4	**66.2**	**63.3**	**61.8**
6	3.487	13.2	17.5	27.5	**32.4**	41.9	49.1	58.9	61.1	**57.6**	**60.7**	**61.7**	65.7	68.2	67.8	**65.4**	**62.1**	**60.8**

* Figures printed in heavy type indicate soaking in formaldehyde; others in water.

as the writer (7) in earlier work has found to maintain with other grains in the presence of gram molecular solutions of sodium chloride. From the consistent behavior of these curves we must conclude that formaldehyde penetrates the coat of wheat, although such entry is slow. The work of BAKKE and PLAGGE (10) offers interesting confirmation of these conclusions. In their work the rate of entry of water and of 1–320 formaldehyde was compared. After a dip of fifteen minutes they found the weight increase about the same for the two, and concluded that water entry from 1–320 is not greatly different from the absorption of distilled water. The question of the comparative entry of water and of formaldehyde solutions becomes specially interesting in the light of COLLINS' (18) work with barley, which indicated that the entry of solutions, and hence the seat of selectivity, is in the germ end of the grain.

DIASTASE

In order to determine the effect on starch digestion of the presence of formaldehyde, a series of twenty-six test-tubes was filled each with 10 cc. of 25 per cent soluble potato starch solution. To all but two of the tubes 1 cc. of a filtered solution of Merck's medicinal diastase of 0.5 per cent concentration was added. To the test-tubes was then added 4 cc. of a formaldehyde solution varying in concentration from 1–1000 through 1–400, 1–320, 1–240, 1–160, 1–80, 1–40, 1–20, 1–10, 1–1, and pure 40 per cent commercial formaldehyde solution. Each condition was run in duplicate. These test-tubes were then incubated for 1.25 hours at 40° C. It was presumed at the beginning of these tests that it would be essential to determine the percentage of reducing sugars as a measure of the degree of digestion. It was found, however, that by modifying the methods used by APPLEMAN (3) and SHERMAN (48), it was easily possible to detect comparative differences in the amount of digestion by the deepness of coloration of the solution upon the addition of iodine. The stock solution of iodine as used eventually by dilution 10 cc. to 100 cc. of water was prepared with 1 gm. iodine, 5 gm. potassium iodide, and 50 cc. water. In the series enumerated the gradation of color was so obvious, from the deep blue of the check to the clear solutions where digestion was complete, that the

experiment was tried of giving to each tube at the end of the test a number value. No digestion (as in the tubes lacking diastase) was indicated by 10, complete digestion (no starch) by 0, and intermediate shades proportionately in between. It is not claimed that this method equals the accuracy of colorimetric technique, yet the differences were so pronounced that on checking over results with

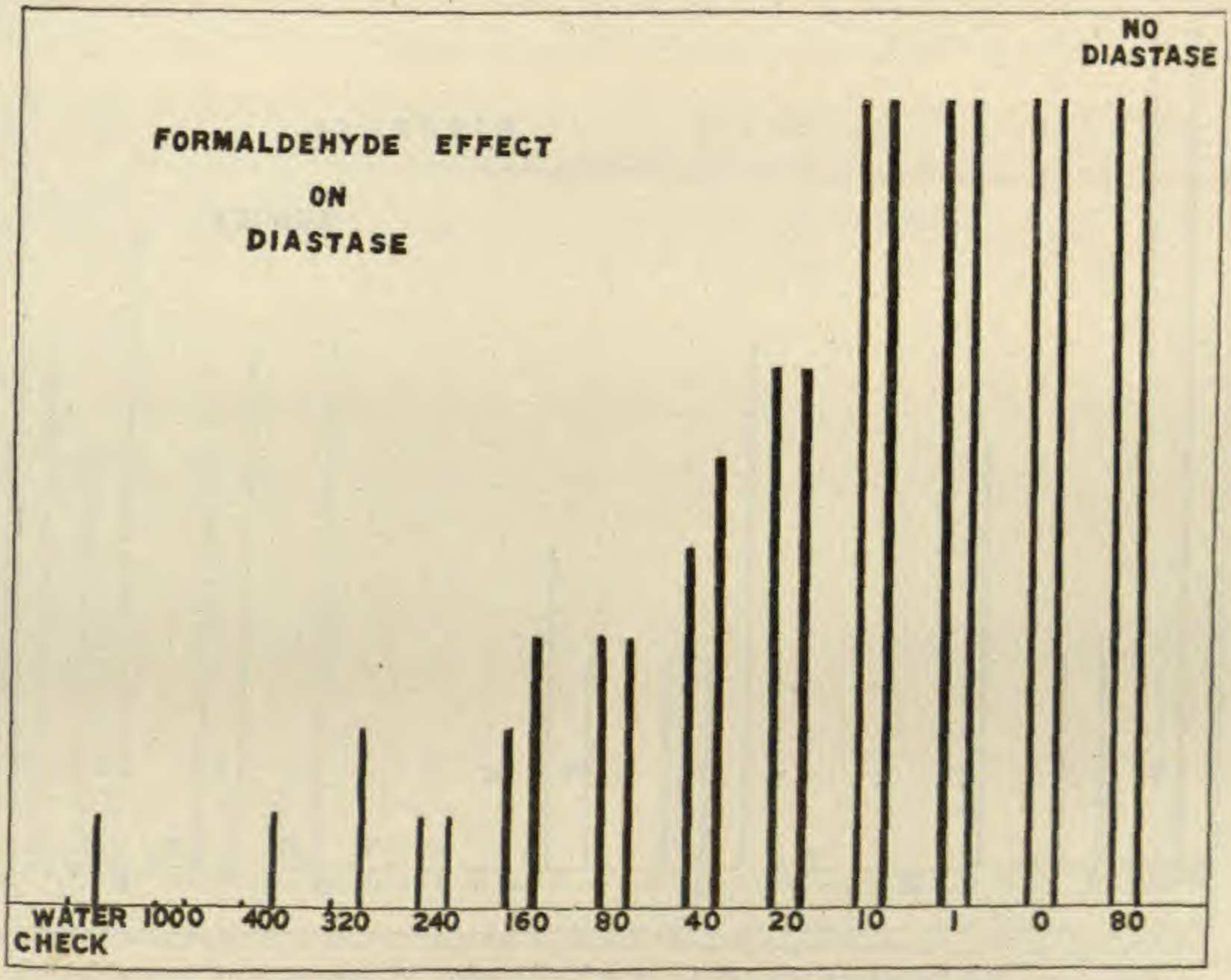

FIG. 6.—Effect of varying concentrations of formaldehyde on starch digestion: height of lines indicates amount of starch remaining undigested (all conditions shown in duplicate); 1 cc. 0.5 per cent solution Merck's diastase, 10 cc. 25 per cent soluble starch solution, and 4 cc. of varying concentrations of formaldehyde used in all but controls.

other observers who were unfamiliar with the conditions presented, it was thought that the situation did not justify the other method. Referring to fig. 6, in which these values are presented graphically, it will be observed that with the higher concentrations of formaldehyde the digestion is not greater in amount than that occurring in the check containing no diastase. Commencing with the concentration of 1–20 of formaldehyde, and running from that point down to

1–400, there is increasingly greater digestion found (less starch remaining). The question immediately arose as to whether the result was inhibition or a retardation of the rate of digestion. This was answered by running two series of digestions over a period of four hours at the same temperature as in the previous tests. To

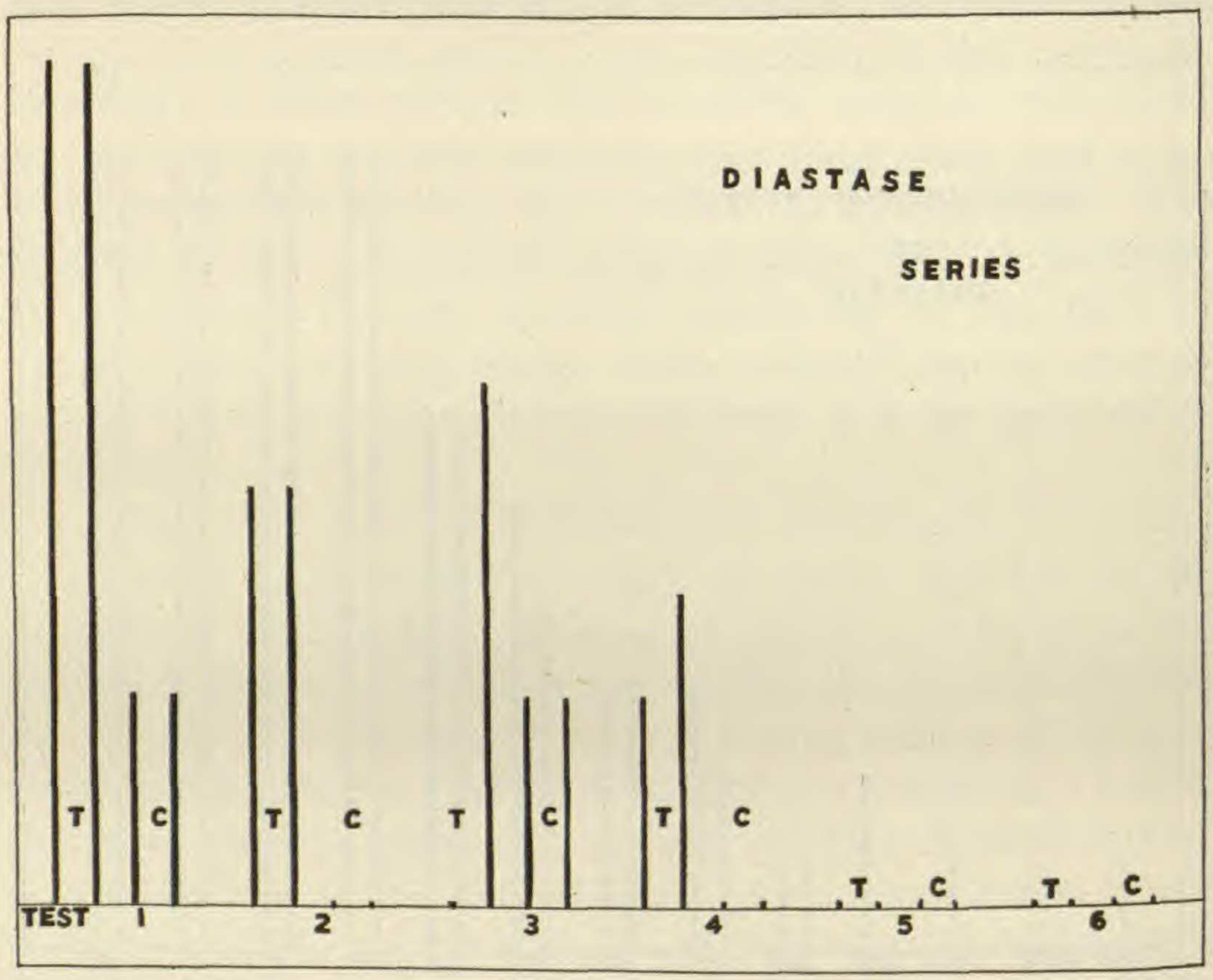

FIG. 7.—Time factor as related to starch digestion in presence of formaldehyde mixed 1–40 parts water: height of line indicates amount of starch remaining undigested (all conditions shown in duplicate); 1 cc. 0.5 per cent solution Merck's diastase, 10 cc. 25 per cent soluble starch solution, and 4 cc. formaldehyde used in tests (*T*), and a like amount water in controls (*C*); time periods in hours tests 1 to 6 respectively, 1.25, 1.5, 1.75, 2, 3, and 4 hours.

all the test-tubes of one series was added 1 cc. of the diastase solution, to one-half of the tubes was added 4 cc. of formaldehyde 1–40, and to the other half (control) an equal amount of water. Ten cc. of the 25 per cent soluble starch solution was placed in all tubes. The second series was prepared in the same manner, except that formaldehyde of 1–320 concentration was used. Four test-tubes from each series were removed every fifteen minutes, two contain-

ing formaldehyde and two controls, and the iodine test applied and results evaluated. Figs. 7 and 8 show that although digestion is markedly checked by 1–40 formaldehyde at the end of the first one and a quarter hours, digestion proceeds with further intervals of time, so that by three hours the digestion which had been com-

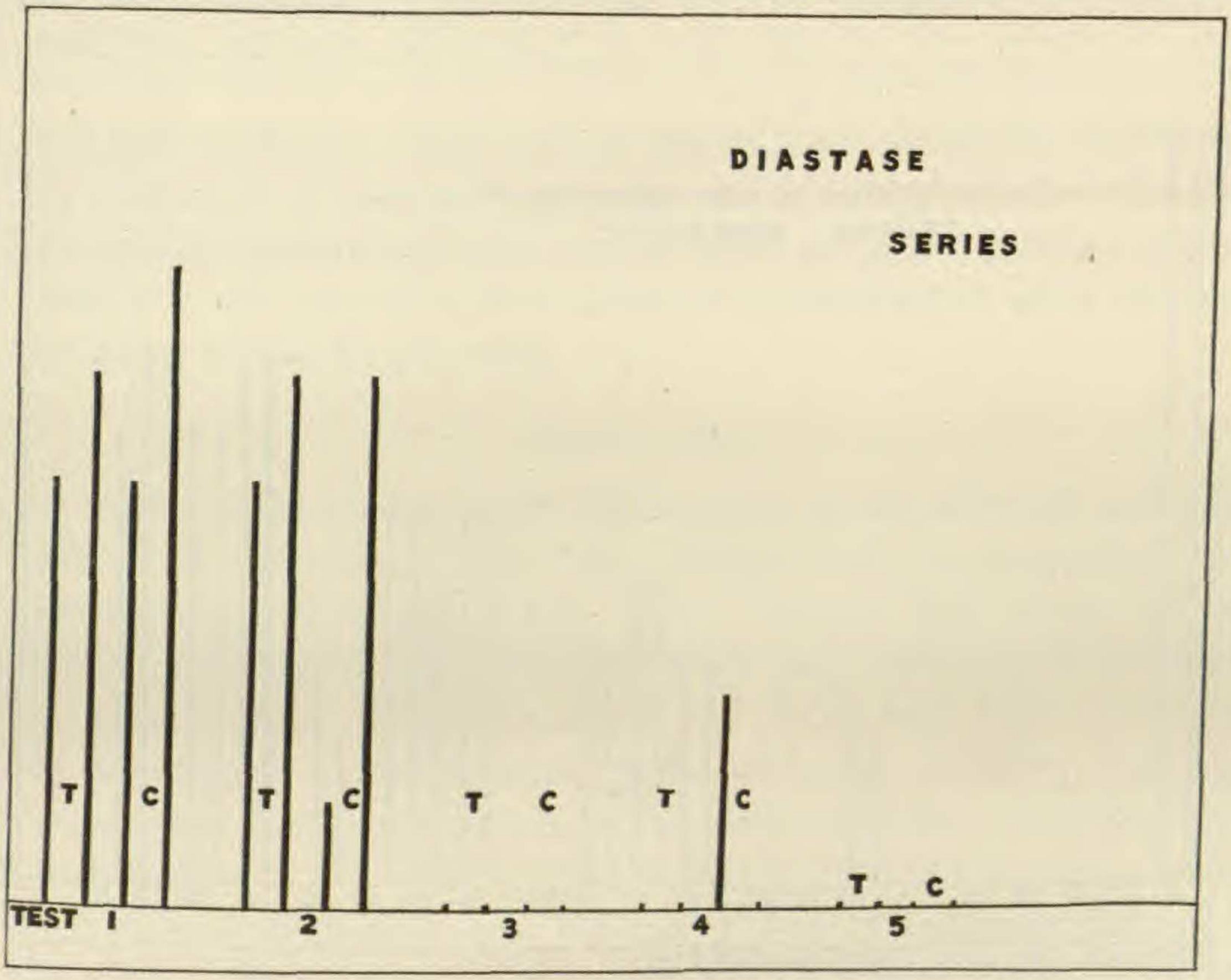

FIG. 8.—Time factor as related to starch digestion in presence of formaldehyde mixed 1–320 parts water: height of line indicates amount of starch remaining undigested (all conditions shown in duplicate); 1 cc. 0.5 per cent solution Merck's diastase, 10 cc. 25 per cent soluble starch solution, and 4 cc. formaldehyde used in tests (*T*), and a like amount of water in controls (*C*); time periods in hours tests 1 to 5 respectively, 1.25, 1.5, 1.75, 2, 2.5 hours.

plete in the controls in half that time, has also occurred in the presence of the formaldehyde. The series in the presence of 1–320 (fig. 8) formaldehyde was not so striking, but nevertheless shows satisfactorily that formaldehyde does not entirely inhibit the action of diastase, but retards the same. Turning to the effects on the starch digestion in living wheat of the concentrations of formaldehyde 1–320, 1–240, 1–160, and 1–80, a considerable quantity of

grain was treated to each concentration ten minutes, allowed to drain, stand moist for two hours, and then thoroughly air dried before an electric fan. The grain was then thoroughly ground in a mill, and extracts of 8 gm. lots made in 100 cc. of redistilled water. Ascending quantities of the water extract were then added to test-tubes each containing 5 cc. of soluble starch prepared as for the

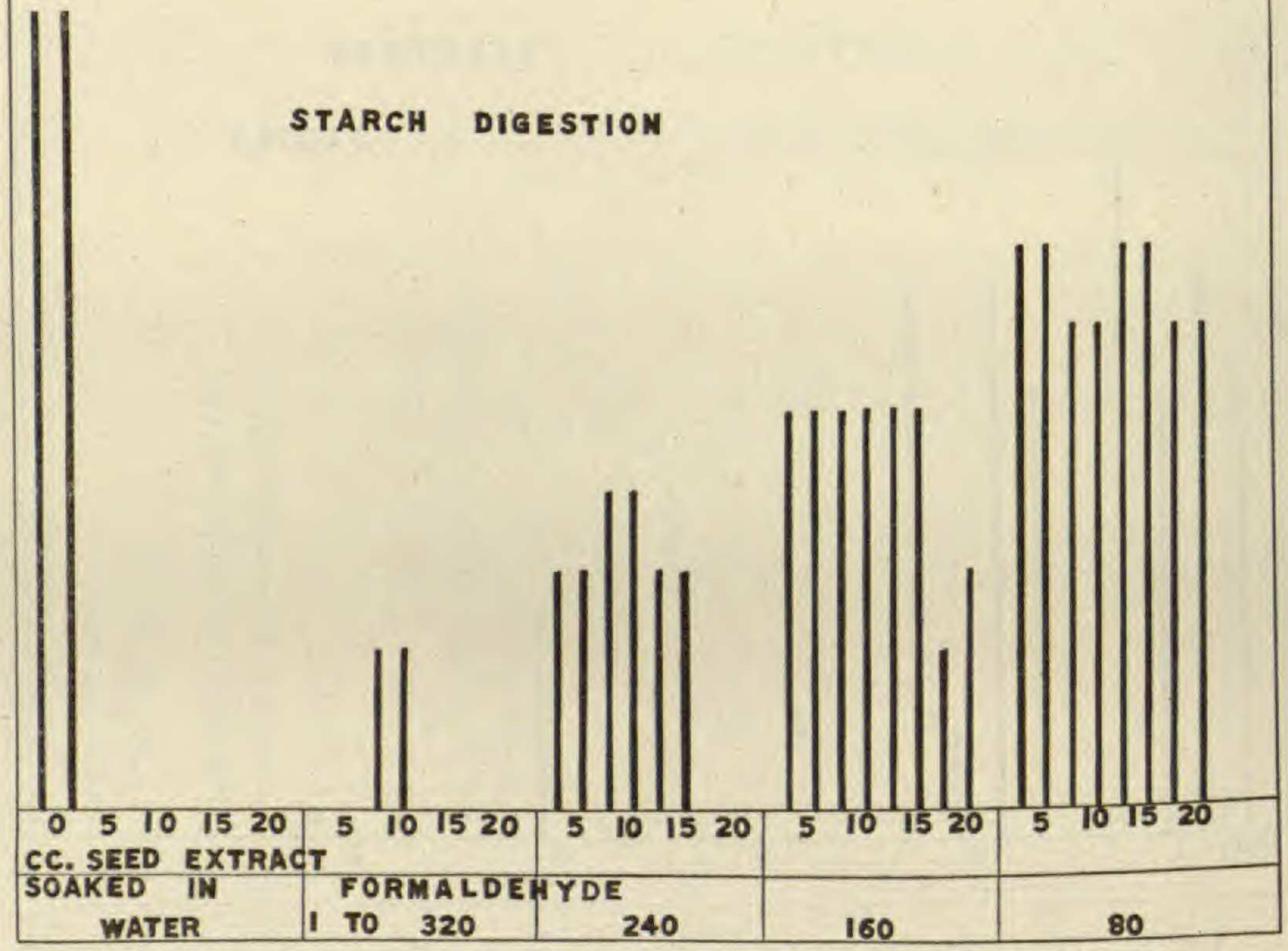

FIG. 9.—Effect of formaldehyde treatment on diastatic activity of wheat extract, seeds treated 10 minutes; concentration of formaldehyde used in treatment varied, and also cubic centimeters of seed extract used: height of lines indicates amount of starch remaining undigested after incubation one hour at 40° C.; 5 cc. 25 per cent soluble starch solution used in each test (all conditions shown in duplicate).

other tests. After incubation for one hour at 40° C. complete digestion had occurred in the controls containing 5, 10, 15, and 20 cc. respectively of the extract of untreated seed. Fig. 9 shows that despite a few unexplained irregularities, the general trend is obviously a reduction in the amount of starch digestion, with a rise in the concentration of the formaldehyde originally used in treatment of the seed. This holds for 5, 10, 15, and 20 cc. seed extract tests

under all the conditions. From these data it would seem certain that treating wheat with formaldehyde retards the availability of carbohydrate to the germinating seedling. BOKORNY (12), reviewing the work of NEUBERG, in which variations showed in the inhibitory effects of different concentrations of formaldehyde on various enzymes and of the effects of the same concentration on different enzymes, explains differences in behavior on the theory of the molecular structure of the enzymes causing different linkages with the formaldehyde. Inasmuch as enzymes are commonly known to be associated at least with proteins, and as formaldehyde is known to react quantitatively with amino acids, as in the Sörensen titration, it is not surprising that effects of formaldehyde upon enzyme behavior should be observed.

AMINO ACIDS

In the light of the results of the tests on the diastatic activity effects of formaldehyde, it was thought well to determine the relationships to amino acids. Miss CHOATE (17) found amino acids occurring in ungerminated wheat and increasing in amount on germination. Miss ECKERSON (27) found only slight amounts of asparagine in the ripened grain, although histidine, leucine, asparagine, and arginine occurred during ripening. The chemical constitution of the wheat grain involves several linked amino acids according to OSBORNE (42), while ABDERHALDEN and SAMUELY (1) in a list of the amino acid constituents of gliadin of wheat flour give alanine, tyrosine, and glutamic acid as among those highest in amount.

As a preliminary test, known quantities of pure amino acids in water solution were determined by the Van Slyke method, both with and without the presence of varying amounts of formaldehyde. Alanine was secured from the organic laboratories of the Eastman Kodak Company, while glutamic acid hydrochloride and tyrosine were purchased from the Special Chemicals Company of Highland Park, Illinois. Careful checking through over 200 tests indicates that such linkages as are formed by formaldehyde and amino acids are broken by the Van Slyke process, and no reduction in nitrogen yields occurred incident to the presence of formaldehyde. The

common use of the formol titration in the determination of amino acids is based on our knowledge that such linkages do occur. It had been assumed that in case such a combination between formaldehyde and amino acids of the germinating seedling does occur the nitrogenous nutrition might easily be disturbed. It is very much hoped that the opportunity may be afforded later to check further upon this point, and also to determine the comparative amount of amino acids liberated in autolysis of treated and untreated seeds.

RESPIRATION

Much effort has been expended in the determination of the effects of seed treatment upon the respiration. PEIRCE and co-workers (43) correlated germinative vigor with respiratory activity. Carbon dioxide has often been recognized as a measure of the activity of the metabolism in the tissues liberating the gas. It was desired to determine whether varying concentrations of formaldehyde, showing varying effects on viability, also affected carbon dioxide releasal in the same manner; in other words, whether the measure of formaldehyde injury may be had by the comparatively accurate carbon dioxide measurements.

Seed lots of 75 gm. each in duplicate were soaked in water as a check, and lots in duplicate in the varying concentrations of formaldehyde, period of soaking being ten minutes, after which they were drained and sealed in respiratory chambers submerged in a constant temperature bath at 28° C. for two hours before beginning the determination. Large museum jars were used for respiratory chambers, equipped with ground glass tops with openings for two-holed stoppers. The seeds were suspended on wire gauze six inches above the bottom of the chamber, while the tubing by which the gases were withdrawn from the chamber during the tests extended to the bottom of the jars. The water bath was 1.5 by 3 feet, and deep enough to permit the tall museum jars to be completely submerged in the water. Under the water bath were placed six porcelain resistance units connected to the lighting system of the laboratory. About 6 feet of small bore glass tubing was bent so as to be submerged in the bath, and filled with mercury, which served to conduct current from two gravity cells to a telegraphic

relay, which at the desired temperature turned off or on the heat under the bath. This arrangement permitted control of the temperature within 0.2° C. Careful checking of the temperature at different points in the bath indicated that stirring devices were not necessary, other than the convection currents from the bottom of the bath upward. Two chambers were used for water soaked wheat (controls), two for the treated wheat, and two blanks to permit checking against leakage.

After setting up the apparatus completely and before making a determination, each of the six complete trains was tested by suction as to its ability to hold up a column of mercury 10 inches high without small leaks permitting the column to settle back again. During the tests a gentle stream of air freed from carbon dioxide was drawn through each outfit for the entire period of hours of the run. In order that the rate of aeration might be uniform in the various outfits, and ample to provide for several complete changes of the air in the respiratory chambers during the course of the experiments, the suction secured from a water pump was conducted to the various chambers through tubing, connected to manometers in such a way that after careful calibration of the separate manometers, the rate of air flow could instantly be determined by a glance at the height of the paraffin oil surface in the manometers. Gas meters of this type were developed in connection with the chemical investigations incident to the recent gas warfare work, and are described in detail by BENTON (11). For each of the six trains air was drawn respectively through 50 per cent potassium hydroxide, a *U*-tube of moist soda lime, and through a barium hydroxide indicator to detect any failure of previous absorbents to remove all carbon dioxide. Air entered the respiratory chamber at the top and was removed from the bottom under the wheat arranged as described. The air then containing the carbon dioxide released by the wheat was drawn immediately through a bead tower containing fourth normal barium hydroxide, out and over another barium hydroxide indicator before passing to the tube connected to the water pump.

The amount of suction was regulated by ground glass stopcocks between the pump and the last indicator flask. With the six com-

plete trains to provide for, it was not deemed practicable to apply the type of automatic pipette arrangement used by BAILEY and GURJAR (9). Instead, a large bottle was thoroughly cleaned and aerated with carbon dioxide-free air and filled with the standard alkali. This reservoir was connected by tubing with a burette, and communication with outside air protected by soda lime traps. At the top of each of the bead tower columns was placed a separatory funnel guarded by a soda lime trap. Before each running the required number of cubic centimeters of the alkali were run directly into the separatory funnel previously washed free of carbon dioxide.

When the whole outfit was ready to make a running, carbon dioxide-free air was run for a sufficient period through the bead tower column to remove all carbon dioxide, before admitting the alkali from the separatory funnel directly into the bead column. This method of determination of carbon dioxide is essentially that described by TRUOG (54). Varying periods of aspiration in these measurements were employed, although experience showed that most satisfactory results could be obtained by employing a period of from four to five hours. No results were considered worthy of recording for second runs of any one lot of samples, as experience showed the need of extreme care to avoid introducing errors incident to the growth of saprophytes upon the check samples, particularly if they were retained at 28° C. longer than one day. BAILEY and GURJAR (9) allowed their moist wheat to stand several days before removing the stagnant air for carbon dioxide determination. Had they used the temperature of 28° C., and had their seed possessed a moisture content of 35–43 per cent, as was the case in these tests, it would have been impossible to avoid questioning the secondary factor of saprophytes which these experiments showed increased tremendously the output of carbon dioxide. Disregarding the fact that they failed entirely to keep their chambers aerated during the course of their work, however, it must be said that they incubated their seeds at 37.8° F., and worked with seeds of moisture contents much lower, in general between 12 and 20 per cent. NABOKICH (41) concluded that part of the carbon dioxide obtained in plant respiration is incident to the same microorganisms that vegetate on leaves and seeds. It was hence a source of much

concern to avoid any fluctuations in respiratory results incident to the gaseous exchanges of saprophytes which might easily be confused with the results of seed treatment. NABOKICH, however, determined that the respiration of microorganisms on seeds may be disregarded during the first day, counting from the time of wetting the seeds. It is thus believed that the data given here eliminate the errors incident to such secondary factor.

Throughout the work over forty runnings were made, representing over 160 different seed lots. As regards the possible criticism that the several per cent variation in moisture content based on dry weight might make the results incomparable, it must be borne in mind that absolute carbon dioxide yields of different runnings are not to be compared with each other, but only the four lots used in any one run. Careful analysis of the variations in carbon dioxide output as related to moisture content has indicated that these variations may not be ascribed to moisture content differences, these observations being made in duplicate independently for the four seed lots of any one running. Fig. 10 and table II summarize the entire results of the investigations on respiration. In all of the work care was taken to have present in the flasks and bead towers at least twice as much of the alkali as would be neutralized by the carbon dioxide liberated by the seeds during any one run. The graphs are expressed in terms of the percentages of the barium hydroxide neutralized. In each case 25 cc. of fourth normal barium hydroxide was used, and at the close titrated against fourth normal hydrochloric acid in the presence of phenolphthalein. If, for instance, 12.5 cc. was neutralized the graph would express 50 per cent values.

Fig. 10 shows marked depression of the respiratory rate for the highest concentrations (1–80) as compared with the water soaked controls. The depression of the respiration rate is evident, although decreasingly so, at 1–160, 1–240, and down to 1–320, the concentration usually used in seed treatment. At 1–400 and 1–1000 the difference between the controls and the treated samples was neither so great nor so constant as to indicate any marked effects of the formaldehyde on metabolism. Special care was used in checking out the situation at the 1–320 concentration, at which point

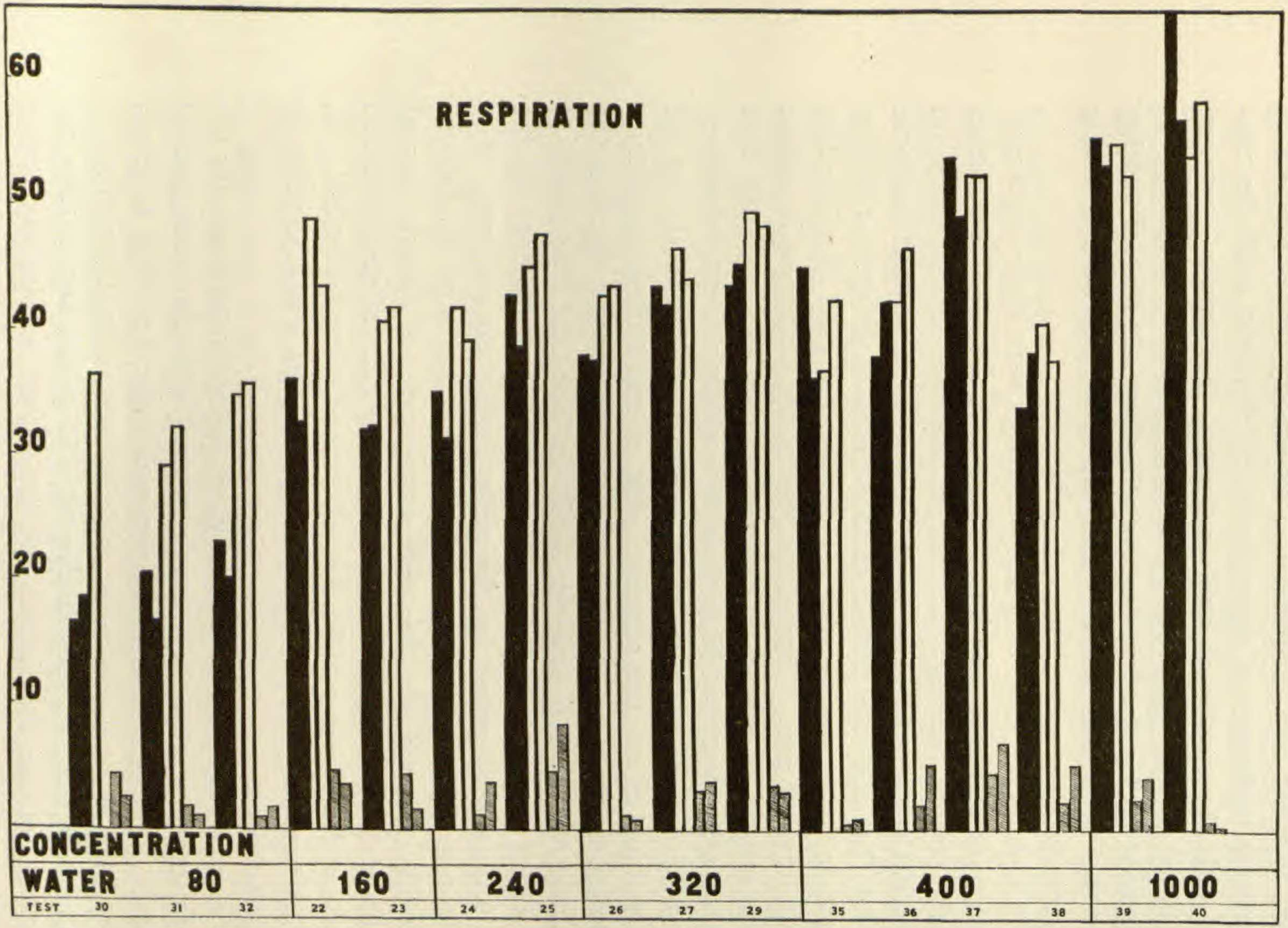

FIG. 10.—Respiration of wheat as influenced by treatment with formaldehyde of varying concentrations; results expressed in terms of percentage of neutralization of total number of cubic centimeters of alkali by carbon dioxide released by seeds; absorption in each case in 25 cc. N/4 barium hydroxide solution; six determinations for each test shown: solid black bars indicate carbon dioxide trapped from treated seeds, white bars indicate magnitudes for water-soaked control seeds, short bars with cross-lining indicate carbon dioxide from empty controls (error).

TABLE II

DATA ON RESPIRATION TESTS AS SHOWN IN FIG. 10

		Test															
No. of test			30	31	32	22	23	24	25	26	27	29	35	36	37	38	39	40
Hours in respirometer before beginning test			2	2	24	2	2	2	2	2	2	2	5.5	2	2	2	3	24.5
Hours test			4.5	4	4	5	4	4.75	5	4.25	5.5	5	4.5	5	5	4	5.5	4
Hours seeds stood moist after treatment			2	2	2	2	2	2	2	2	2	2	5.5	2	2	2	3	24.5
Parts water to one part of formaldehyde			80	80	80	160	160	240	240	320	320	320	400	400	400	400	1000	1000
Treated wheat	Percentage moisture at end of test	1	40.15	38.85	38.85	.....	42.25	36.09	36.92	37.90	39.30	39.38	39.20	38.70	40.50	.....	43.60	43.60
		2	39.20	36.85	36.85	.....	40.23	35.25	37.70	39.70	38.80	37.60	35.65	36.35	38.80	.....	41.00	41.00
	Cc. barium hydroxide combined	1	4.10	5.11	5.72	8.95	7.97	8.95	10.59	9.45	10.77	10.81	11.16	9.44	13.36	8.48	14.02	16.33
		2	4.63	4.16	5.01	8.12	8.03	7.76	9.58	9.34	10.44	11.17	8.95	10.48	12.21	9.50	13.17	14.12
	Percentage cc. hydroxide combined	1	16.48	20.52	23.00	36.10	32.10	35.10	42.70	38.10	43.42	43.60	44.90	37.92	53.70	33.91	55.14	65.70
		2	18.60	16.72	20.14	32.72	32.40	31.30	38.65	37.62	42.10	45.10	36.10	42.20	49.10	38.20	53.00	56.80
Water-soaked control	Percentage moisture at end of test	1	.....	36.40	36.40	.....	41.60	36.40	37.00	41.00	37.30	37.80	37.88	39.90	38.40	.....	40.00	40.00
		2	37.40	36.85	36.85	.....	39.67	35.20	40.18	41.48	40.00	.....	36.60	42.28	39.48	.....	38.45	38.45
	Cc. barium hydroxide combined	1	.....	7.24	8.63	12.14	10.08	10.37	11.15	10.59	11.55	12.25	9.13	10.44	12.97	10.03	13.61	13.33
		2	9.03	8.01	8.91	10.75	10.40	9.72	11.80	10.82	11.14	11.98	10.53	11.51	13.01	9.33	12.98	14.41
	Percentage cc. hydroxide combined	1	.....	29.10	34.70	48.90	40.70	41.80	45.00	42.70	46.60	49.4	36.70	42.00	52.20	40.38	54.70	53.63
		2	36.30	32.20	35.80	43.4	41.90	39.2	47.6	43.6	44.95	48.40	42.40	46.30	52.30	37.5	52.20	58.00
Empty controls (error)	Cc. barium hydroxide combined	1	0.65	0.48	0.26	1.19	1.15	0.30	1.17	0.60	0.72	0.90	0.13	0.48	1.16	0.56	0.61	0.18
		2	1.13	0.28	0.46	0.92	0.42	0.95	2.12	0.20	1.00	0.78	0.08	1.33	1.76	1.31	1.06	0.08
	Percentage cc. hydroxide combined	1	2.61	1.93	1.05	4.80	4.64	1.21	4.72	1.14	2.90	3.63	0.52	1.93	4.66	2.25	2.45	0.72
		2	4.54	1.13	1.85	3.71	1.70	3.83	8.55	0.81	4.03	3.15	0.32	5.35	7.08	5.27	4.26	0.32

evidently the concentration is near the border line of injury, since 1–400 does not definitely display such uniform depression of respiration. It was desired to compare the respiratory rates of young seedlings from treated and untreated wheat, but as yet technique has not been devised which avoids the luxuriant development of *Rhizopus* in the warm moist atmosphere of the respirometer. In these tests, as in those with the seeds alone, special care was used to avoid air infection of the respirometers. Just previous to a running, the interior of each jar was wiped out with cotton moistened with mercuric chloride (1–1000). The further precaution was taken of flaming the gauze on which seeds and seedlings were placed. KARCHEVSKI, as quoted by BAILEY and GURJAR (**9**), found the energy of wheat respiration as measured by carbon dioxide releasal twelve times greater for the embryos than for the entire seeds. This seems to indicate that the data may be more largely influenced by factors affecting the embryos than otherwise. The facts that formaldehyde denatures proteins, that the embryo is rich in proteins, and that the respiration data show the effects of formaldehyde treatment, make it possible that the injurious effects of formaldehyde are intimately connected with injury to the embryo itself. This is in harmony with the findings of COLLINS (**18**), in a study of the coat of the barley grain, that the entry of solutions and hence the seat of selectivity is in the germ end of the grain.

It is of interest in this connection to note that although Miss HURD (**33**) believed the injurious effects of formaldehyde to be attained by a slow absorption of the gas liberated from paraformaldehyde, and although these studies of the penetration of formaldehyde do not show any sudden penetration of the fungicide, nevertheless within a period of time as short as three to six hours during respiratory determinations, some effect is exerted upon the seed which very definitely modifies the respiratory rate as compared with water soaked controls. One can but wonder whether here, as in the case of the studies of CROCKER and KNIGHT (**24**), we may not have in plant responses a more delicate indicator of injury than are the chemical reactions commonly used in detecting these injurious substances.

Catalase

The work of recent years has shown that there often seems to be a relationship between respiration of plant tissues and the catalase content. Appleman (**4, 5**) has shown this relationship in the case of potatoes and corn, Crocker and Harrington (**23**) in the case of seeds. The latter workers find that this relationship is not universal, for while imbibed Johnson grass has its respiration paralleled by the catalase activity, this is not true for the seeds of *Amaranthus*. The most interesting observation is further made that in the case of Johnson grass where this parallelism is found, neither the respiratory activity nor the catalase content is paralleled by the vitality of the seeds or the seedlings. Inasmuch as seed vitality and seedling vigor are definitely related to formaldehyde treatment, and this work has linked in also the effects upon respiration, it was thought to be of interest to determine whether any effects of seed treatment could be noted upon the catalase activity of wheat.

Catalase activity in wheat was determined much after the method suggested by Appleman (**2**), and later employed with various modifications by other workers (**17**). Two series of experiments were conducted, one to see whether any effects of formaldehyde could be noted immediately after treatment while the grain was still moist, and another to see whether the effects of treatment persist on grain which has been treated and air dried before an electric fan in the laboratory and subsequently exposed for about a month to the air of the laboratory. The concentrations of formaldehyde used were 1–80, 1–160, and 1–320. About 1 gm. of air dried wheat was used in each case, weighed on the analytical balance, and results computed to the basis of 1 gm. For a reaction chamber a bottle of 250 cc. capacity was used and shaken continuously during the ten minutes of the test by a mechanical shaker making 129 excursions per minute. The reaction chamber was submerged in the constant temperature bath previously described, and was kept at 28° C. Dioxygen was used and neutralized with calcium carbonate. It was found that 3 mg. of the chemically pure salt used would neutralize 5 cc. of the peroxide, and this proportion was observed throughout. The gas evolved was run into a 100 cc. gas burette,

and all readings corrected for temperature and barometric pressure, the equivalent corrected volumes at 0° C. and 760 mm. being computed for 1 gm. sample. Ten cc. water and 5 cc. peroxide as neutralized were used.

In the case of the freshly treated seeds, they were soaked for ten minutes in the formaldehyde solutions, and kept moist for two or more hours, until tested. The moisture of the seeds precluded

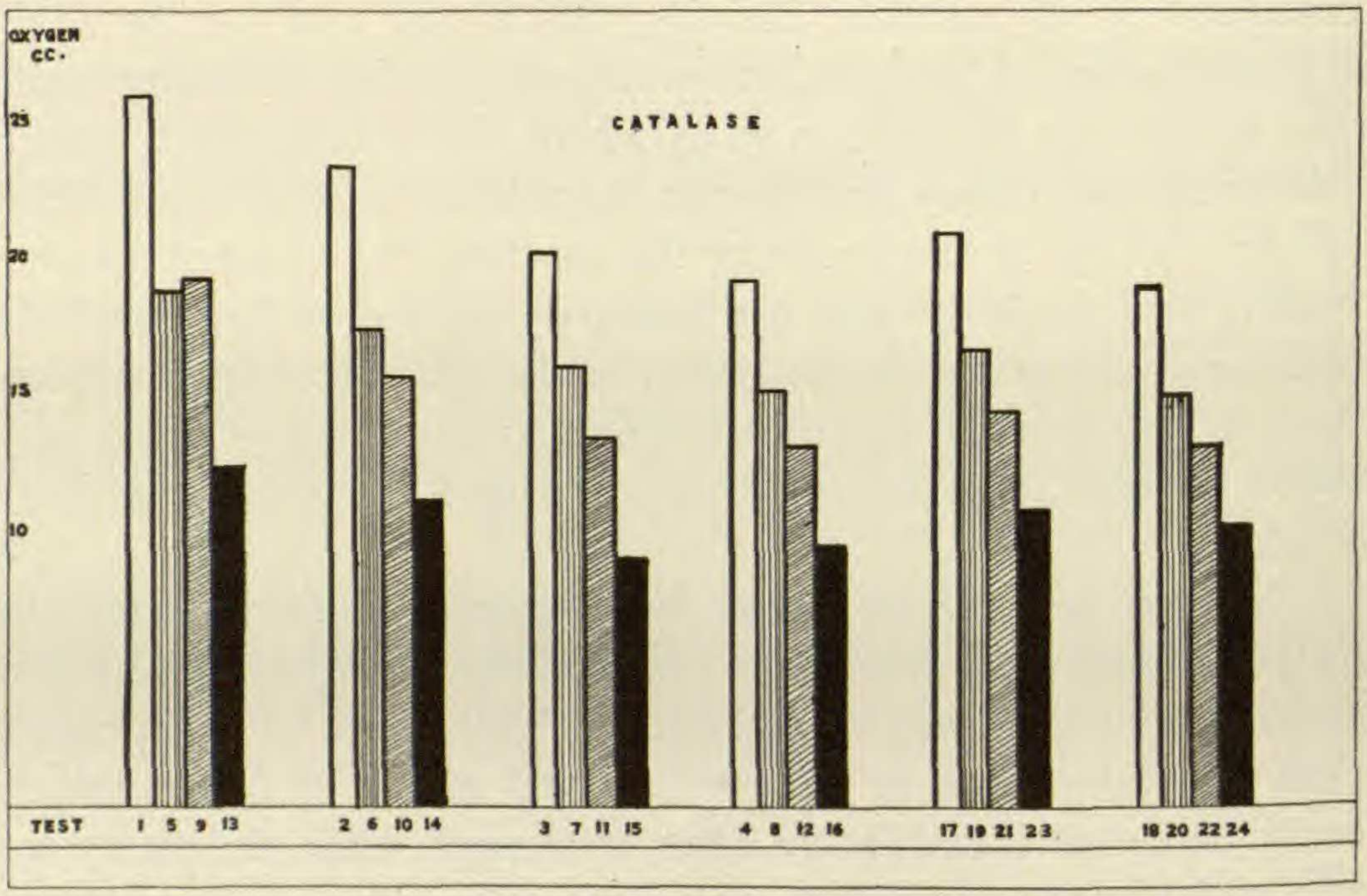

FIG. 11.—Effect of formaldehyde in varying concentrations upon ability of seed extract to liberate gaseous oxygen from dioxygen: height of lines indicates cubic centimeters of oxygen computed for 1 gm. samples and constant temperatures and pressures; extract from water-soaked seeds (controls) indicated by white, vertical lining indicates extracts from seeds treated in formaldehyde 1–320, diagonal lining formaldehyde 1–160, and solid black formaldehyde 1–80; in all cases seeds tested while still moist.

the possibility of passing the material through bolting cloth (23), but it was soon found that a material difference in the crop of oxygen given off resulted if the seed material was more or less glutinous and forming sticky masses, or dry enough to separate fairly well on grinding in a mortar with carborundum as an abrasive; hence all surface moisture was removed with filter paper before grinding up the seeds. Inasmuch as differing moisture contents of the differing lots would occur between the first lots tested and the last in a long series, the errors incident to this cause were avoided

as far as possible by testing consecutively the control seeds and those treated with 1–320, 1–160, and 1–80. Such a series of four naturally fall together for comparison in one group, and many such series were made, the combined results of which may easily be seen from fig. 11 and table III. With some small variations, the results were surprisingly uniform in showing the steady depressing effect of

TABLE III

DATA ON CATALASE TESTS AS SHOWN IN FIG. 11

Test no.	Concentration formalin	Weight of sample	Volume oxygen released	Barometer	Temperature	Hours between treatment and test	Corrected volume equivalent of 1 gm. sample
1	Water control	1.015	30.1	748	24	2	25.9
2	Water control	1.005	26.9	748	24	3.5	23.4
3	Water control	1.006	23.5	748	25	7	20.3
4	Water control	1.009	22.4	748	25	8.5	19.3
5	1–320	1.008	21.8	748	24	2.5	18.9
6	1–320	1.000	20.1	748	24	3.75	17.5
7	1–320	1.004	18.6	748	25	7.25	16.1
8	1–320	1.002	17.6	748	25	9	15.3
9	1–160	1.006	22.2	748	24	2.75	19.3
10	1–160	1.015	18.3	748	24	3	15.8
11	1–160	1.007	15.7	748	25	7.5	13.6
12	1–160	1.002	15.3	748	25	9	13.3
13	1–80	1.010	14.4	748	24	3	12.5
14	1–80	1.011	13.0	748	24	3	11.3
15	1–80	1.006	10.6	748	25	8	9.20
16	1–80	1.010	11.2	748	25	9.5	9.63
17	Water control	1.017	24.6	747	24.5	5	21.1
18	Water control	1.012	22.3	745.5	25	7.75	19.1
19	1–320	1.007	19.4	747	24.5	5	16.8
20	1–320	1.010	17.7	745.5	25	8.25	15.2
21	1–160	1.003	16.7	747	24.5	5.25	14.5
22	1–160	1.011	15.5	745.5	25	8.5	13.3
23	1–80	1.003	12.7	747	24.5	5.5	11.0
24	1–80	1.013	12.1	745.5	25	8.75	10.4

formaldehyde on catalase activity. As the concentration of the formaldehyde rose, the catalase activity as measured by oxygen yield fell.

In the studies of the effects of formaldehyde on catalase activity after the seeds had been dried about a month in the laboratory, comparison was made only between the control and the seeds treated in 1–80 formaldehyde. Fig. 12 and table IV show that there is a definite depressive effect of the treatment on catalase activity, but it is by no means so great as soon after treatment.

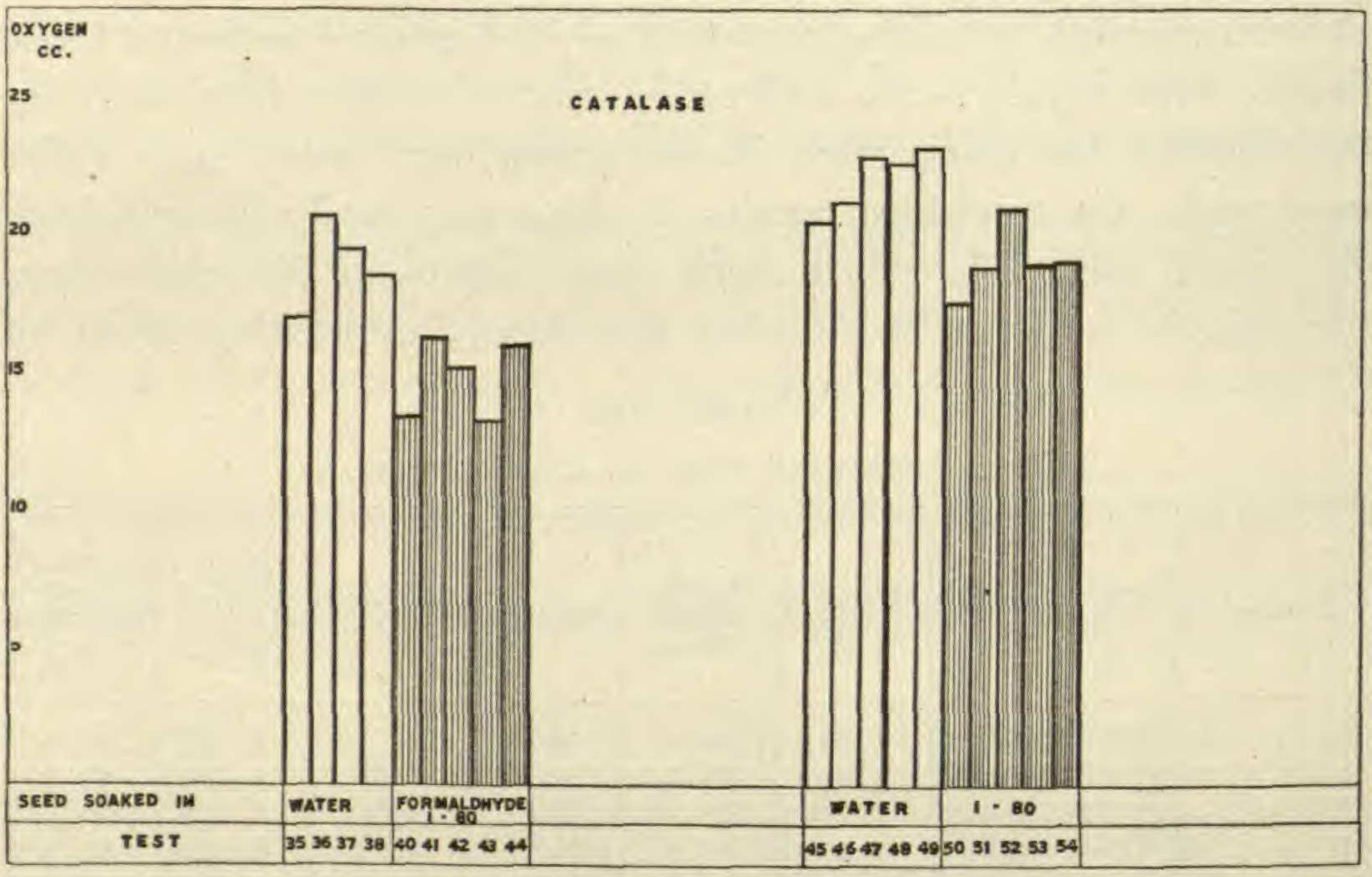

FIG. 12.—Effect of formaldehyde 1 to 80 upon ability of seed extract to liberate gaseous oxygen from dioxygen: height of lines indicates cubic centimeters of oxygen computed for 1 gm. samples and constant temperatures and pressures; extract of water soaked seeds (controls) indicated by white, treatment in 1–80 indicated by vertical ruling; in all cases seeds tested after thoroughly drying one month in laboratory after treatment.

TABLE IV

DATA ON CATALASE TESTS AS SHOWN IN FIG. 12

Test no.	Concentration formalin	Weight of sample	Oxygen (cc.)	Barometer	Temperature	Corrected volume equivalent of 1 gm. sample
35.....	Water control	1.014	20	743.5	25	17.10
36.....	Water control	1.002	24	743.5	25	20.7
37.....	Water control	1.005	22.5	743.5	23	19.5
38.....	Water control	1.006	21.5	743.5	24	18.6
40.....	1–80	1.014	15.7	743.5	24	13.5
41.....	1–80	1.005	18.8	743	23	16.3
42.....	1–80	1.002	17.5	743	23	15.2
43.....	1–80	1.020	15.5	743	23	13.3
44.....	1–80	1.000	18.5	743	23	16.1
45.....	Water control	1.011	23.5	745	21.5	20.5
46.....	Water control	1.011	24.2	745	20.5	21.2
47.....	Water control	1.012	26.1	745	20.5	22.9
48.....	Water control	1.014	25.9	745	21	22.6
49.....	Water control	1.014	26.7	745	21.5	23.2
50.....	1–80	1.006	20.0	745	21.5	17.6
51.....	1–80	1.003	21.3	745	20.5	18.8
52.....	1–80	1.002	23.8	745	21	21.0
53.....	1–80	1.011	21.8	745	21.5	19.0
54.....	1–80	1.001	21.7	745	21.5	19.1

This would seem to support the view that the injury is due more to exterior members retaining the formaldehyde which had been volatilized in part, than to a permanent injury to the embryo having resulted from the treatment.

General considerations

The treatment of seeds with fungicides is a process wherein one plant tissue (that of the parasite) must be destroyed, while another tissue (that of the seed) must be conserved. It is entirely probable that the points of fungicidal effectiveness and of danger to seeds are not far separated. DE ZEEUW (26), in noting this point, quotes work in which it was found that the seeds were more sensitive to formaldehyde than spores of either bacteria or fungi, when its action was deeper than the surface, as is essential to secure sterile seeds. He believes that the high concentrations of disinfectants required to care for the destruction of bacterial spores is explained by the protection afforded by the seed coat on which the spores are lodged. There is an interesting similarity between this statement and the findings of REIMER (46), who observed that in the control of the bacillus producing fire blight of pears, a disinfectant which is serviceable upon tools is ineffective when used upon the organic substrate of the wood of the tree itself.

It would seem that we have been too ready to jump to conclusions and give "recommendations" as to treatment upon the basis of germination data of more or less extent. In the light of the preceding results, it seems doubtful whether it is safe to postulate the boundary lines of safe and dangerous concentrations merely upon the basis of germination data. STEPHENS (50) has emphasized the relationship of seed treatment to subsequent lowered vitality of seedlings. It is thus entirely possible that concentrations which do not materially injure germination percentages do materially disturb the physiological processes related to germination and subsequent growth. Common agricultural practice and the findings of the War Emergency Board of American plant pathologists seem to indicate that 1–320 is at the edge of the danger zone, if indeed such zone is not here passed. If, as Miss HURD (33) believes, a polymer of formaldehyde is deposited on dried treated wheat, and subse-

quent injury to the grain is incident to the liberation of formaldehyde gas from the precipitate, with its resultant solution in the moisture content of the living cells, the process must be a slow cumulative one which is entirely in harmony with the definite although slow entry of formaldehyde herein shown actually to occur. Furthermore, even though the grain be treated with 1–320 formaldehyde, such a deposit of the polymers would result in the presentation to the living cells of a much stronger concentration than that of the dip used. Yet even at the concentration of 1–320 it is evident that while germination is often but lightly affected, the diastatic power of wheat is retarded, the catalases are less active, and respiration is definitely reduced. It is not impossible that such results indicate a decided tendency to a reduction in seedling vitality even in the presence of germination.

It is highly desirable that these studies be pursued further to determine the relationship of treatment to the proteolytic activities of the germinating seed, and further, to determine whether the recommended "presoak" of BRAUN (13) or the "post-washing" of Miss HURD (33) correspondingly modify the physiological activities and alleviate their injury.

Summary

1. Tests of formaldehyde entry into wheat have been made both by microchemical tests and by imbibitional studies, indicating that formaldehyde slowly enters through the seed coat.

2. Diastatic activity of the grain is retarded.

3. Respiration is slowed down.

4. Catalases are reduced in their ability to break down peroxides.

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